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Separation of basic amino acids and related compounds on Aminex A5 resin and elution with a lithium buffer

In a previous paper[1] dealing with a method for the analysis of amino acids and related compounds found in biological fluids by means of a new two-channel automatic apparatus for ion-exchange chromatography[2], the conditions for eluting the basic amino acids and related compounds from a short column of Amberlite IR 120 resin with a lithium buffer, were determined for the first time. Under the conditions described[1] a separation of γ-aminobutyric acid (GABA), tryptophan, creatinine, ornithine, lysine, ammonia, histidine, 1-methylhistidine, 3-methylhistidine and arginine could be obtained in 120 min. It should be mentioned that such a separation in such a short time is not feasible on Amberlite IR 120 resin when it is eluted with a sodium citrate buffer.

However, in the daily routine use of this method, it has been observed that the separation obtainable for the ornithine–lysine doublet often does not permit a good evaluation of ornithine, which usually is present in a quantity which gives a much smaller peak than the subsequent lysine. In such cases it only appears as a shoulder on the lysine peak. All attempts to improve this resolution, such as variations in working temperature, buffer pH and ionic strength, elution flow rate, apparent volume of resin, etc. have been unsuccessful.

The results obtained by using a Bio-Rad Aminex A5 resin in the lithium cycle on our analytical system for the separation of the above-mentioned compounds are reported in this note.

Experimental

The buffer employed had the same composition as that described for the operation with Amberlite IR 120 crushed resin, *i.e.* lithium concentration, 0.6 *M*; citrate concentration, 0.2 *M*; lithium citrate·4 H_2O, 56.40 g; concentrated HCl, 17.5 ml; 10% Brij solution, 2 ml; phenol, 1 g; final volume, 1000 ml. The pH had to be adjusted to 4.52 in order to achieve the best resolution and this was obtained by adding HCl. Brij was added just before using the buffer and the pH value given is that of buffer which does not contain this surfactant. It should be mentioned that the addition of Brij is not strictly necessary.

The column employed had a total length of 30 cm, an internal diameter of 0.7 cm and a resin bed height of 15 cm, *i.e.* an apparent bed volume of 5.8 ml. The thermostating water in the column jacket was kept at 55.2° for all the analysis time, as against 53° and 65° necessary for eluting Amberlite IR 120 crushed resin.

The eluting buffer flow rate was reduced to 1 ml per min and consequently the ninhydrin colour reagent was pumped at 0.5 ml per min. At these flow rates the working pressure measured at the top of the column did not exceed 10 kg/cm^2.

According to the criteria reported in a previous paper[3], the amount of resin was chosen so as to have the least apparent bed volume, which, for the indicated flow rate, allowed the separation of the components with a resolution pattern which did not impair the degree of accuracy and precision required. The column diameter for this given amount of resin and this buffer flow rate was chosen taking into con-

sideration the optimum conditions of working pressure; as a matter of fact, under these conditions, the progressive packing of the resin and the consequent pressure rise does not take place.

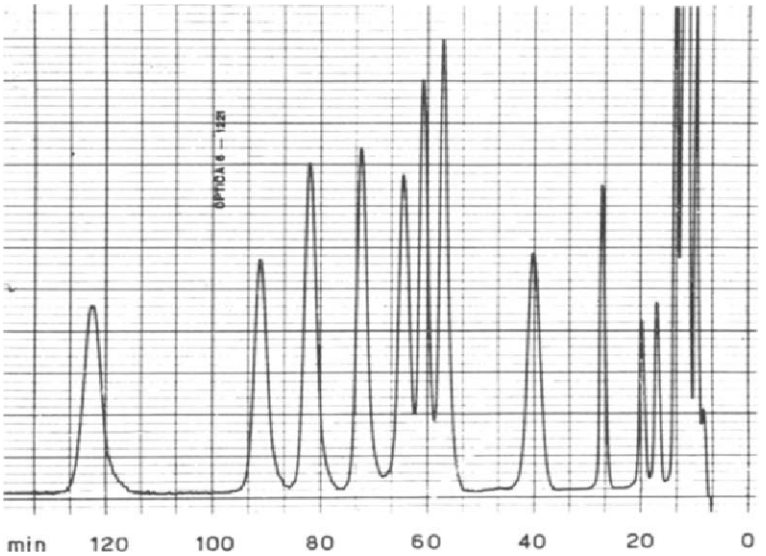

Fig. 1. Separation of amino acids. From right to left: tyrosine, phenylalanine, GABA, tryptophan, ornithine, lysine, ammonia, histidine, 1-methylhistidine, 3-methylhistidine and arginine.

Results

In Fig. 1 a chromatogram obtained by loading the column with 0.75 ml of a calibrating mixture containing 18.75 nmoles of tyrosine and phenylalanine, 37.5 nmoles of GABA and 75 nmoles of tryptophan, ornithine, lysine, ammonia, histidine, 1-methylhistidine, 3-methylhistidine and arginine is shown. The total chromatography time is less than 130 min. Consequently, if analysis is started on our system after the proline peak has been recorded on the first channel, the chromatogram of the basic amino acids and related compounds and the chromatogram of the acidic and neutral ones are complete at the same time. The optical system, reading at 570 nm, was provided with a flow cuvette having a 10-mm light path and a 2-mm internal bore, and the sensitivity was set at 1 O.D. unit for a full scale deflection of the recorder pen. The chart speed on the recorder was set at 3 in./h. The pen-recording on the chart is linear with respect to the optical density, and it should be mentioned that this new system allows peak evaluation simply by measurement of the height in millimetres above the base-line with a precision higher than that achieved by measurement of their areas[1,2].

Should particular experimental conditions require a higher resolution pattern, this can be achieved: (a) by reducing the buffer flow rate; (b) by increasing the amount of resin; and (c) by both increasing the amount of resin and reducing the buffer flow rate. As already demonstrated in a previous paper[3], in the first case the working pressure will get lower and the sensitivity will increase, in the second case the working pressure will increase and the sensitivity will diminish, and in the third case these two parameters may remain unaltered.

In addition a study of the effects of temperature and pH changes upon the resolution pattern given by Aminex A5 resin when employed in the lithium cycle

for this chromatographic separation was carried out. Of all the basic amino acids and related compounds which have been taken into consideration, the most sensitive to variations of these parameters is lysine. It was observed that either an increase in the pH of the eluting buffer or an increase in the working temperature accelerates the elution of lysine, so that overlapping of the peaks of lysine–ammonia diminishes while the overlapping of ornithine–lysine peaks increases. An opposite effect is obtained when a diminution of one or both of these parameters occurs.

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